1385-Pos Board B277
Super-Resolution Imaging of AKAP79/150 Signaling Complexes using Stochastic Optical Reconstruction Microscopy (STORM)
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Our previous FRET and functional studies suggest AKAP (A-kinase-anchoring-protein) 79/150 to be crucial in modulation of ion channel activity and neuronal function, by orchestrating important molecules such as protein kinases, phosphatases, G protein-coupled receptors and ion channels into signaling complexes in the plasma membrane. In this study, to directly visualize these AKAP79/150 signaling complexes and interactions between AKAP79/150, ion channels and receptors in sympathetic neurons, we utilized stochastic optical reconstruction microscopy (STORM) with sub-diffraction (~20 nm half width) resolution. STORM uses dyes that can cycle between a dark and a fluorescent state thousands of times, thus enabling detection of the precise localization of the center of these scattered spots given by cumulative integration of each cycle. Consistent with previous immunostaining/confocal studies, STORM imaging of fixed Chinese hamster ovary (CHO) cells using fluorescently labeled antibodies against AKAP150, or KCNQ2 and KCNQ3 subunits revealed the precise plasma membrane localization for the former, and membrane and cytoplasmic distribution of the latter, at the single-molecule level. using multi-color STORM to simultaneous image AKAP150, KCNQ2-3 and $G_{q/11}$-coupled muscarinic M_1 receptors, further experiments on sympathetic neurons isolated from AKAP150+/+ or AKAP150-/- mice will shed light on the spatial co-distribution of AKAP150 with its signaling partners, and whether loss of AKAP150 causes redistribution of ion channels and receptors.

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Use of Voltage-Sensitive Phosphatase to Investigate the Location of the PIP2-Binding Site on KCNQ2 Channels
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Phosphatidylinositol 4,5-bisphosphate (PIP2) is known to be necessary for the activation of KCNQ K^+ channels. Many studies have investigated the location of the PIP2-binding site on those channels, which have strongly implicated binding to the C-terminus. Our lab has suggested the linker between helices A and B in the C-terminus of KCNQ2 to be the primary site of PIP2-binding, with K452, R459 and R461 shown as critical (Hernandez et al., 2008, JGP, 132). However, other labs have reported PIP2 interactions to be dependent on basic residues at the start of the C-terminus (Zhang et al., 2003, Neuron, 37; Thomas et al., 2011, JBC, 286). A histidine at 328 position in KCNQ2 and four residues (K354, K358, R360, and K362) in KCNQ1 have been suggested to interact with PIP2. Since three of those four residues are conserved in KCNQ2-5 channels, we investigated a potential role of those residues in PIP2-channel interactions for KCNQ2. We found mutations at analogous positions in KCNQ2 (K319A, R325A and K327A) to decrease whole-cell current amplitudes. However, use of a voltage-sensitive phosphatase from danio rerio, which dephosphorylates almost all PIP2 upon strong membrane depolarizations, revealed the PIP2 affinity of the K319A and K327A mutants to be increased, rather than decreased, as assayed by the rates of current decay upon depolarization to 120 mV, and subsequent current recovery at 30 mV. These results indicate that the decrease of KCNQ2 currents for all the mutants is likely not due to a lower PIP2 affinity for the channels. Indeed, unlike KCNQ1, charge neutralization mutations at the start of the C-terminus seem to augment PIP2-binding to channels, consistent with the previously-indentified linker between helices A and B to be the primary PIP2-binding site in KCNQ2 channels.

Ligand-gated Channels I

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Bulky Substitutions in the Thumb Domain of ASIC1a Restrict Proton-Gating
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Acid-sensing ion channels (ASICs) are trimeric proton-gated cation selective ion channels expressed in the nervous system. Each monomer consists of two transmembrane helices with short cytoplasmic tails and a large extracellular region organized in distinct domains named the thumb, finger, knuckle, β-ball, and palm. In this report we investigated the contribution to mouse ASIC1a activation and desensitization of three pairs of acidic residues, Glu238-Asp345, Asp237-Asp349 and Glu219-Asp407. Residues in each pair are located in close proximity and together form an acidic cluster at the interface between the thumb and β-ball domains. We examined the reactivity toward methanethiosulfonate (MTS) reagents of channels carrying individual Cys substitutions at these positions. MTSET-modification of D345C channels shifted the pH of half-maximal activation (pH50) from 6.23 ± 0.06 to 4.48 ± 0.08, and increased the desensitization rate. A similar effect was observed with other positively-charged MTS reagents such as MTSMT, MTSPT and MTSEA, as well as with MTSES, a negatively-charged reagent. Moreover, Lys substitution at position 345 shifted pH50 toward more acidic values. Remarkably, Lys substitution at positions 219, 237 or 238 did not significantly change the pH50, suggesting that acid residues at these positions do not substantially contribute to proton activation. Our results indicate that the introduction of a bulky moiety at position 345 restricts conformational movements required for proton-gating, suggesting that the thumb-β-ball interface undergoes dynamic rearrangements during activation.

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Cleft Closure Mechanism in Gating of Acid Sensing Ion Channel 1A
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Acid sensing ion channels (ASICs) are trimeric proton activated channels which share sequence similarities with Degenerin/Epithelial sodium channels. They are responsible for proton evoked currents in central nervous system of most vertebrates. Their physiological functions include synaptic transmission, sensory perception, learning, and pathophysiology of stroke. Crystal structure of the low pH state of chicken ASIC shows thumb and palm like domains in the extracellular part of each subunit connected through hydrogen bonds between carboxylate residue pairs, thought to be part of proton sensors in ASIC. Based on the low pH structure it's been hypothesized that these domains would be further apart at high pH due to negative charges and protonation would allow the two domains to move closer, which in turn could trigger changes in the transmembrane segments, causing channel opening. We have used Fluorescence Resonance Energy Transfer(FRET) to study the high pH resting structure of ASIC and determined conformational changes between the thumb and palm domains. Cysteine residues were introduced on these domains and tagged with thiol reactive donor and acceptor fluorophores. The recognition site for protease Xa was introduced on either side of the cysteine on the palm domain. This allowed us to perform the FRET measurements in oocytes and HEK-293 cells without purification, as addition of the protease allowed us to quantitively characterize the background FRET. The FRET constructs were characterized using electrophysiology to establish that they were functional. The FRET based distances showed 3Å decrease in distance between thumb domain residue 340 and palm domain residues 130 and 139 upon changing the pH from 8 to 6. This decrease in distance provides evidence for the hypothesized movement in the extracellular domain of ASIC and also supports the protonation of the carboxylate pairs across these domains.

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An Acidic Ring in the Palm Domain of ASIC1a Facilitates Pore opening
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Acid-sensing ion channels (ASICs) are neuronal cation selective proton-gated ion channels that contribute to nociception, mechanosensation, synaptic plasticity, learning and memory, and fear conditioning. ASIC subunits have two transmembrane helices, intracellular N- and C- termini, and a large extracellular region organized in discrete domains named the thumb, finger, palm, beta-ball and knuckle. The palm domain is directly connected to the pore-forming transmembrane helices. We combined site-directed mutagenesis and electrophysiology to examine the contribution of residues Glu79 and Glu416 to ASIC1a proton-gating and desensitization. These acidic residues are located in the core of the palm domain forming a ring-like structure. Individual substitution at these positions to Cys, Lys or Gln shifted the apparent proton affinity of ASIC1a approximately one pH unit toward more acidic values. A similar shift in apparent proton affinity was observed in channels bearing substitutions at both positions, indicating that Glu79 and Glu416 cooperatively contribute to proton-gating. E79C and E416C mutants were modified by MTSET in the closed, but not in the desensitized state. MTSET-modification of E79C increased the magnitude of the response to protons by approximately six-fold and slowed desensitization by ten-fold, while MTSET-modification of E416C only reduced the response to protons nearly 50 percent. Ala mutagenesis at neighboring positions of Glu79 indicates that the covalently modified side chain of Cys at position 79 became closer to residues in the thumb and palm domains during channel gating. Our results suggest that Glu79 and Glu416 facilitate pore opening, and that the palm domain experiences a rotation during proton-dependent activation and desensitization.